published his *Handbuch der Biochemie* in which he offered the following characterization:

> Biochemistry is the science that deals first with the constituents of living tissues, and their determination, properties, and reactions; but which also strives to draw from the chemical changes that proceed during life processes, conclusions regarding the scope of the life-process itself. (Oppenheimer, 1909, pp. v–vi)

Historians have proposed two major factors as differentiating the newly emerging biochemistry from the physiological chemistry of the nineteenth century that I have been describing. Robert Kohler (1973) emphasized the strategy of identifying different enzymes responsible for each reaction.[32] As we have just seen, investigators had already identified a host of catalysts responsible for physiological operations before Kühne coined the term, and by the 1890s physiologists generally accepted that digestion in animals was the work of enzymes. Emil Fischer's (1894) research and his lock-and-key model of enzyme action had revealed the highly specific nature of enzyme action. All of the reactions for which enzymes had been discovered to that point, however, were simple hydrolytic ones (that is, they consumed water in reactions that split apart organic substances). What Kohler emphasized is that until the 1890s no catalysts had been identified as involved in the more complex reactions of cells such as respiration or fermentation and none had been demonstrated to operate within cells (all enzymes known at that time were secreted by cells and operated outside).

Kohler presented Gabriel Bertrand (1895) as having taken the first step beyond hydrolytic enzymes when he determined that a catalyst he identified in the Chinese art of making black lacquer finish, laccase, caused the uptake of oxygen and the production of carbon dioxide. Bertrand compared the process to artificial respiration.[33] The key step in demonstrating that enzymes could operate within cells came with Eduard Buchner's (1897) report (discussed below) that in a cell-free extract made by grinding yeast cells with sand, fermentation still occurred. Appealing to enzymes rather than protoplasm to explain processes in living organisms, according to Kohler, marked the major change from physiological chemistry to biochemistry: "the very language of

[32] Kohler cites Franz Hofmeister (1901) as giving voice to the view that all reactions could be explained by enzymes: "we may be almost certain that sooner or later a particular specific ferment will be discovered for every vital reaction" (p. 14).

[33] Kohler identified a second major expansion of the notion of enzymes, occurring with Arthur Croft Hill's (1898) discovery that maltase, in appropriate circumstances, synthesized maltose from glucose rather than cleaving maltose to form glucose.